tackle this challenge and are in the process of developing microfluidic assays for PTMs that would complement protein interactions. Our goal is to established a multi-dimensional microfluidic platform that can recapitulate a biochemical pathway in vitro in both the interaction and PTM dimensions. Here, I will present our advance toward this goal. We have established an assay to detect specific Tyr phosphorylation and dephosphorylation. A second advantage obtained with this assay is the ability to take 'snapshots' of physiological conditions by flowing cell extracts onto the microfluidic device. Another advantage of the microfluidic approach is that we can gain high-throughput while still being quantitative. In the future, we plan to expand the arsenal of modifications and expand the platform into proteomic-scale screens.

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Tuning Microbial Surfaces to Control Carbonate Mineralization

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Carbon dioxide sequestration is an essential component of climate change mitigation, including geologic sequestration the underground storage CO_2, and surface sequestration. Carbonate minerals are stable forms of CO_2 storage, but their geologic formation is slow. Many microbes alter carbonate mineral morphology; however mechanisms of such mineralization are largely unknown. Hypothesized mechanisms include metabolic processes that alter pH and supersaturation, as well as cell surface properties that induce mineral nucleation. This work systematically investigates these mechanisms by allowing calcium carbonate ($CaCO_3$) formation in the presence or absence of microbes with various native surfaces features including lipopolysaccharides, surface layer proteins (S-layers) and engineered surface features.Surprisingly, formation of stable crystalline $CaCO_3$ was accelerated by the presence of all microbes relative to abiotic solutions. This rate acceleration also occurred for metabolically inactive bacteria, indicating that metabolic activity was not necessary. Rather, since $CaCO_3$ crystals increased in number as the cell density increased, results indicate that many bacterial species accelerate the nucleation of $CaCO_3$ crystals. To understand the role of specific biomolecules on nucleation, we engineered variants of surface layer proteins (S-layers), which are tractable models to evaluate surface interfaces through peptide display. Bacterial surface charge and cation binding was assessed and correlated to bacterial surface chemistry and biomineralization experiments. From these results, we propose that the S-layer surfaces that can selectively attract Ca^{2+} ions, serve as nucleation sites for $CaCO_3$, thereby accelerating crystal formation, and that engineered variants with selective cation binding enhance this effect. These observations provide substantive evidence for a non-specific nucleation mechanism, and stress the importance of microbes, on the rate of formation of carbonate minerals. This work also indicates that microbes with tuned S-layer surfaces could be used to enhance the sequestration of CO_2 as stable mineral carbonates. http://foundry.lbl.gov/afgroup/index.html

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De Novo Design of Bioactive Protein-Resembling Nanospheres via Dendrimer-Templated Peptide Amphiphile Assembly

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Peptide amphiphiles (PAs) self-assemble into nanoparticles that can resemble proteins in both structure and function, and are highly sought after for a litany of applications ranging from disease diagnostics to therapeutics. However due to their propensity to form large or high aspect ratio particles such as vesicles or cylindrical micelles, respectively, their use is limited where small spherical particles are preferred. Here we report a modular approach for directing the self-assembly of biorelevant PAs into spherical, sub-100 nm structures using dendrimers designed with hydrophobic peripheries as supramolecular templates. Two PAs that naturally form large aggregates were employed as models. Specifically, di-C16-bZip, a DNA binding PA sequence, which self-assembles into micron-long cylindrical micelles, and di-C16-NLS, a cell internalization PA, which self-assembles into polydisperse vesicles of 100 to 400 nm in diameter, were used.

The dendrimer templating approach was successful directing the self-assembly of both PAs to form 50 nm spherical particles, which were designated "protein resembling templated nanospheres" (PRTNs). The dendrimer directed assemblies were significantly more stable when compared to their PA-only counterparts, indicated by a decrease in their critical aggregation concentration and their enhanced stability in the presence of serum proteins. bZip peptides displayed in the PRTNs were in their native helical conformation, and DNA binding studies showed that the peptide retained activity. 50 nm PRTNs possessing both DNA binding and cell internalizing functions were formed by mixing di-C16-bZip and di-C16-NLS, and their co-assembly was confirmed by FRET. These particles were bio-compatible and were internalized by cells through an endocytic pathway. Having developed a reliable approach to control the assembly of PAs, we seek to further employ this versatile and modular method for the fabrication of synthetic multifunctional protein-like particles from the bottom-up.

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Freeze-Fracture Electron Microscopy on Nano- and Microcarrier for Theranostics and Antibiotics

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Nano- and microparticles are frequently used as carrier systems for delivering therapeutics as well as diagnostics (theranostics) at cellular level. We studied the morphology of a wide variety of such particles suitable as theranostic-carrier by freeze-fracture transmission electron microscopy (ff-TEM). As a cryofixation, replica, and transmission EM method it is a powerful tool to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules [1]. At the resolution limit of 2nm we are able to characterize nano particles such as quantum dots (coupled to drug-loaded immunoliposomes), gold nano-particles, superparamagnetic iron oxide nano-particles loaded in polymeric immunomicelles, micelles (spherical-, disc-, and worm-type micelles), single-wall carbon nano-tubes embedded in hydrated gels, and small unilamellar liposome (targeted and non-targeted) on nano-scale resolution. Furthermore, ff-TEM allows the production of beam-damage-resistant replica from much larger, micrometer-size objects such as multilamellar liposome, niosomes, cationic liposome/DNA complexes, integrin-targeted lipopolyplexes, polymer-, lipid- or surfactant-stabilized gas bubbles, cochleate cylinder [2], depofoam particles, and drug crystals. This way we are able to study nano-scale events in micro-scale biological as well as artificial assemblies. Ff-TEM enables us not only to characterize nano- and microcarriers suitable for theranostics, but is the method of choice as well to study their fate related to their pay load, application milieu, and during cell interaction. Furthermore, we investigated structural modifications within bilayers such as domain-formation [1] but also transformations to non-bilayer structures such as hexagonal and cubic phases. Currently we are focused on cochleate cylinder as macrocarrier for antibiotics combating bacterial multidrug resistance [2].

References

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Single-Cell Biopsy using Nanopipettes

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To fully understand cell functioning and stochastic gene expression patterns, it necessary to measure molecular interactions at the single cell level. Advances in microscopy as well as genomics technology have enabled detailed analysis of gene expression at the single-cell level, but the means to manipulate individual cells are still limited. Nanotechnology-based tools having high sensitivity and low invasiveness are holding great promises as new biomedical devices for single cell manipulation. The fully electrical read-out as well as the ease and low cost of fabrication are unique features that give nanopipette technology enormous potential. We developed a single-cell manipulation platform based on nanopipettes. The system uses scanning microscopy techniques to position the nanopipette with nanoscale precision, and electro-wetting to aspirate minute amount of cytoplasmic material from individual cells without comprising cell viability. It is well documented that the application of a voltage at the interface across two immiscible liquids changes their surface tension. The resulting force is sufficient to cause liquid to flow into/out of the nanopipette, with displacement volumes much smaller than a typical mammalian cell. Multiple biopsies can be taken from the same cell to study gene expression patterns. We will present preliminary RNA-seq data of material biopsied from a single cell. We envision that this technology will allow biological analysis with unprecedented level of detail, allowing a better understanding of single cell dynamics.

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Engineering Biological Nanopores with Enhanced Properties

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With strong substrate specificity and multitude of control points, biological nanopores are promising devices for developing reagentless DNA sequencing, bio-alarm systems, monitoring single-molecule chemical reactions, bio-inspired